

# Plating Techniques

For agar plates, add 10.0 g of agar per liter of E-MSGM. Add 1% of total volume E-MSGM in Wolfe's Vitamin Solution [ATCC #MD-VS] and 1% of total volume E-MSGM in iron malleate solution.

## Method 1:

Bacteria can be plated on 1% agar directly, as done for E. coli. Plates must be wrapped using an excess of Parafilm M® to reduce oxygen content in environment. Colony formation in 20-24 hours.

## Method 2:

Cells can be plated on 1% agar idish and grown in an anaerobic chamber. Colony formation in 10-12 hours.

### To use the Anaerobic Chamber:

Make sure inner door is closed.

Open outer door, place object in outer chamber.

Close outer door.

Press start button. This starts the cycle - three evacuations (first and second are filled with N<sub>2</sub>, third with mixture of CO<sub>2</sub>, N<sub>2</sub>, H<sub>2</sub>).

When anaerobic light turns on, open inner door and bring objects inside.

Always close inner door when finished.

### 2. To bring something out of the chamber:

Make sure both doors are closed.

Press start button (to exchanged atmosphere in out chamber).

When anaerobic light turns on, open inner door and put plates in out chamber.

**CLOSE INNER DOOR!**

Open outer door and remove objects. Close outer door.